Venovenous perfusion-induced systemic hyperthermia: Five-day sheep survival studies

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Objective: Since hyperthermia selectively kills lung cancer cells, we developed a venovenous perfusion-induced systemic hyperthermia system for advanced lung cancer therapy. Our objective was to test the safety and accuracy of our venovenous perfusion-induced systemic hyperthermia system in 5-day sheep survival studies, following Good Laboratory Practice standards.

Methods: Our venovenous perfusion-induced systemic hyperthermia system, which included a double-lumen cannula (Avalon Elite, Rancho Dominguez, Calif), a centrifugal pump (Bio-Pump 560; Medtronic Inc, Minneapolis, Minn), a heat exchanger (BIOtherm; Medtronic Perfusion Systems, Brooklyn Park, Minn), and a heater/cooler (modified Blanketrol IIIl Cincinnati Subzero, Cincinnati, Ohio), was tested in healthy adult sheep ($n = 5$). The perfusion circuit was primed with prewarmed Plasma-Lyte A (Baxter Healthcare Corp, Deerfield, Ill) and de-aired. Calibrated temperature probes were placed in the right and left sides of the nasopharynx, bladder, and blood in/out tubing in the animal. The double-lumen cannula was inserted through the jugular vein into the superior vena cava, with the tip in the inferior vena cava.

Results: Therapeutic core temperature (42°C-42.5°C), calculated from the right and left sides of the nasopharynx and bladder temperatures, was achieved in all sheep. Heating time was 21 ± 5 minutes. Therapeutic core temperature was maintained for 120 minutes followed by a cooling phase (35 ± 6 minutes) to reach baseline temperature. All sheep recovered from anesthesia with spontaneous breathing within 4 hours. Arterial, pulmonary, and central venous pressures were stable. Transient increases in heart rate, cardiac output, and blood glucose occurred during hyperthermia but returned to normal range after venovenous perfusion-induced systemic hyperthermia termination. Electrolytes, complete blood counts, and metabolism enzymes were within normal to near normal range throughout the study. No significant venovenous perfusion-induced systemic hyperthermia–related hemolysis was observed. Neurologic assessment showed normal brain function all 5 days.

Conclusions: Our venovenous perfusion-induced systemic hyperthermia system safely delivered the hyperthermia dose with no significant hyperthermia-related complications. (J Thorac Cardiovasc Surg 2014;148:2360-6)

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Lung cancer is the leading cause of cancer-related deaths in the United States.[1] Non–small cell lung cancer (NSCLC) accounts for approximately 85% of new lung cancer cases and is often diagnosed at an advanced stage.[2] Patients with advanced NSCLC have only a 9- to 13.5-month median survival.[3-6] Moreover, chemotherapy results in only a 1.5-month improvement in survival over supportive care alone.[7] Thus, a critical need for more effective lung cancer therapies exists.

Hyperthermia is a promising new therapy for advanced lung cancer because lung cancer cells are thermosensitive with significantly reduced heat shock protein expression.[8] Hyperthermia selectively kills lung cancer cells via apoptosis[8-10] and increases the cytotoxicity of chemotherapy. Moreover, hyperthermia reverses cisplatin resistance by enhancing platinum uptake and inhibiting platinum-induced DNA repair.[9-13]

Whole-body hyperthermia for advanced cancer treatment has been proposed since the 1970s.[10,14,15] However, it has not been proven to be clinically practical for cancer treatment in terms of safety and efficiency. We developed an efficient yet safe therapeutic hyperthermia dose (42°C-42.5°C for 2 hours) for cancer treatment.[16-18] Temperatures below this hyperthermia dose do not kill cancer cells, whereas temperatures above it will cause normal cell damage.[10,13,19] Precise control of the whole body temperature to fit this narrow therapeutic hyperthermia window is difficult. A venovenous perfusion-induced systemic hyperthermia (vv-PISH) system was developed to precisely deliver the thermal dose (42°C-42.5°C for 2 hours) for advanced lung cancer treatment. The bulky and complicated

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Abbreviations and Acronyms

ABP = arterial blood pressure
CPK = creatinine phosphokinase
CVP = central venous pressure
DLC = double-lumen cannula
GLP = Good Laboratory Practice
IV = intravenously
NSCLC = non–small cell lung cancer
PA = pulmonary artery
PAP = pulmonary artery pressure
SC = subcutaneously
vv-PISH = venovenous perfusion-induced systemic hyperthermia

first-generation vv-PISH system resulted in unwanted and worrisome clinical consequences, preventing further clinical investigation.[18] For practical clinical application of hyperthermia, we recently developed a simplified vv-PISH system and management protocol.[20,21] We report the results of our preclinical Good Laboratory Practice (GLP) investigation that was designed to prove the safety and accuracy of the simplified vv-PISH system in a 5-day sheep survival study. Our results showed that an accurate dose of therapeutic hyperthermia was safely delivered to sheep with no hyperthermia-related complications.

METHODS

All animal studies were approved by the University of Kentucky Institutional Animal Care and Use Committee, conducted in accordance with the "Guide for the Care and Use of Laboratory Animals," and performed in compliance with GLP standards.[22]

Anesthesia and Instrumentation

Adult female cross-breed sheep (32-36 kg, n = 5) were intubated after anesthesia induction with ketamine (5 mg/kg, intravenously [IV]) and diazepam (0.25 mg/kg, IV), followed by 4% to 5% isoflurane. After intubation, anesthesia was maintained with 1% to 3% isoflurane through the anesthesia machine (Narkomed 2B, DRAGER, Telford, Pa). Prophylactic analgesia (buprenorphine 0.005-0.020 mg/kg, subcutaneously [SC]) and antibiotic (enrofloxacin 7.5 mg/kg, SC) were administered. The sheep were ventilated at 8 to 10 mL/kg tidal volumes with 12 to 20 respirations per minute to maintain 30 to 35 mm Hg end-tidal carbon dioxide.

A Gelli-Roll warming gel pad (Cincinnati Subzero, Cincinnati, Ohio) was placed under the sheep, and a Warm-Air convective warming blanket (Cincinnati Subzero) was placed over the sheep before instrumentation. These external heating devices were set at 38°C to maintain baseline core temperature and set at 42°C during the heating and therapeutic phases to augment hyperthermia. Two 16G catheters (Becton Dickinson, Sandy, Utah) were placed into the femoral artery and vein for blood sampling/pressure monitoring and fluid administration, respectively. A Swan–Ganz catheter (Edwards Lifesciences, Irvine, Calif) was placed percutaneously through the left jugular vein to the pulmonary artery (PA) for measurement of cardiac output, pulmonary artery pressure (PAP), central venous pressure (CVP), and PA blood temperature. The catheters were connected to transducers (Edwards Lifesciences) for monitoring arterial blood pressure (ABP), CVP, and PAP via a Phillips MP-50 monitor (Phillips, Boeblingen, Germany). Temperature probes were placed in the bladder (Foley catheter), right and left sides of the nasopharynx, and blood in/out tubing. To avoid isoflurane-induced vasodilation and consequent lower blood pressure during hyperthermia, the dosage of isoflurane was reduced with the addition of continuous propofol (0.4 mg/kg/min) IV infusion and bolus vecuronium IV injection (loading dose: 0.025-0.4 mg/kg, subsequent doses: 0.007-0.1 mg/kg) to maintain operative anesthesia.

Installation and Maintenance of Venovenous Perfusion-Induced Systemic Hyperthermia

The vv-PISH system consisted of (1) a double-lumen cannula (DLC) (Avalon Elite, Rancho Dominguez, Calif)[23]; (2) a centrifugal pump (Bio-Pump 560, Medtronic Inc, Minneapolis, Minn); (3) a heat exchanger (BIOtherm; Medtronic Perfusion Systems, Brooklyn Park, Minn); and (4) a heater/cooler (modified Blanketrol III; Cincinnati Subzero). The vv-PISH blood circuit was flushed with carbon dioxide and primed with preheated (up to 46°C) priming solution (Plasma-Lyte A; Baxter Healthcare Corp, Deerfield, Ill), which circulated with the heater to maintain the temperature.[21]

Systemic anticoagulation was initiated with a heparin bolus (150 U/kg, IV) and maintained at an activated clotting time of 180 to 250 seconds. The DLC was inserted in the right jugular vein into the superior vena cava, traversing the right atrium, with the tip positioned in the inferior vena cava. The DLC was connected to the primed vv-PISH circuit. When the pump was started, the venous blood was drained from the DLC drainage lumens (inferior vena cava/superior vena cava) and sent to the heat exchanger for heating. The heated blood was pumped back through the DLC infusion lumen into the right atrium–pulmonary circulation. The circuit blood flow was 1.5 to 1.6 L/min to heat the sheep, targeting a 42°C core temperature. This 42°C core temperature was maintained for 2 hours for the therapeutic window. After 2 hours of hyperthermia, the cooling phase

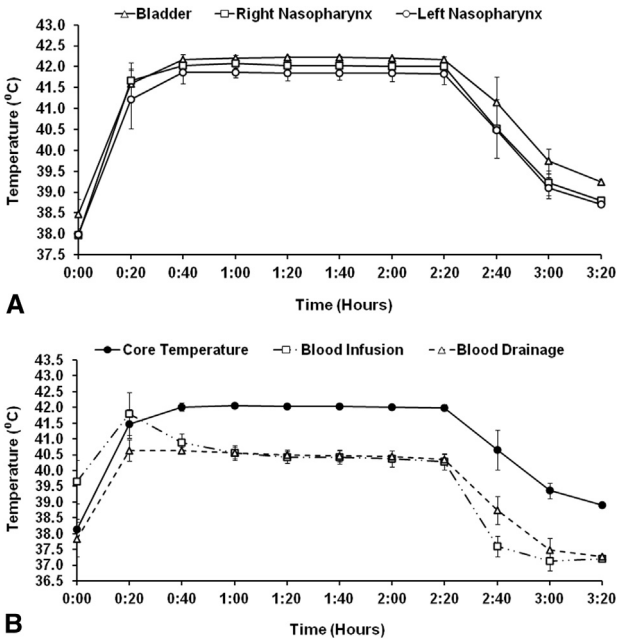

FIGURE 1. Hyperthermia temperature profile. A, Bladder, right/left nasopharynx temperatures measured during heating, therapeutic, and cooling phases. Homogeneous heat distribution was observed with no temperatures greater than 42.5°C. B, Relationship between core temperature and circuit infusion (blood in)/drainage (blood out) temperatures. Maximal circuit blood infusion temperature was 42.3°C.

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TABLE 1. Hemodynamics

	Baseline	Heat 39	Heat 40	Heat 41	Therapy start	Therapy middle	Therapy end	Cool 41
MAP	106 ± 11	98 ± 17	108 ± 10	103 ± 9	99 ± 9	102 ± 5	101 ± 5	105 ± 9
HR	110 ± 16	131 ± 14	150 ± 32	152 ± 26*	153 ± 18*	159 ± 27*	156 ± 21*	154 ± 23*
mPAP	14 ± 5	15 ± 4	15 ± 3	15 ± 4	15 ± 4	14 ± 2	16 ± 3	16 ± 2
CVP	3 ± 2.2	4 ± 3.8	4 ± 4.5	4 ± 4.9	4 ± 5.1	5 ± 3.6	6 ± 2.9	6 ± 2.7
CO	4.5 ± 1.7	ND	ND	ND	8.6 ± 3.3*	10.9 ± 4.3*	10.7 ± 2.7*	ND
PAWP	6 ± 3.0	ND	ND	ND	5 ± 2.5	5 ± 2.3	6 ± 3.1	ND

Hemodynamics were recorded at baseline, heat 39 (core temperature 39°C during heating), heat 40 (core temperature 40°C during heating), heat 41 (core temperature 41°C during heating), therapy start (when 42°C target was met), therapy middle (1 hour at 42°C), therapy end (2 hours at 42°C), cool 41 (core temperature 41°C during cooling), cool 40 (core temperature 40°C during cooling), cool 39 (core temperature 40°C during cooling), day 1 (posthyperthermia day 1), day 2 (posthyperthermia day 2), day 3 (posthyperthermia day 3), day 4 (posthyperthermia day 4), and day 5 (posthyperthermia day 5). N = 5 sheep. *CO*, Cardiac output; *CVP*, central venous pressure; *HR*, heart rate; *MAP*, mean arterial pressure; *mPAP*, mean pulmonary artery pressure; *ND*, not determined; *PAWP*, pulmonary artery wedge pressure. *$P < .05$ versus baseline.

was started by circulating cool water through the heat exchanger until the core temperature returned to 39°C.

Data Acquisition During Venovenous Perfusion-Induced Systemic Hyperthermia

The data-acquisition system used in this study was the cDAQ9172 (National Instruments, Austin, Tex) with temperature, pressure, and flow modules. The temperature module was connected to temperature probes placed in the bladder, right and left sides of the nasopharynx, and blood in/out tubing for constant temperature measurement. The core temperature was defined as the average of the bladder and right and left nasopharynx temperatures. The pressure module was connected to the pressure sensors for ABP, CVP, and PAP monitoring. The flow module was connected to a flow meter (T110, Transonic Systems Inc, Ithaca, NY) for circuit blood flow monitoring. Data acquisition software (Labview 8.6, National Instruments) was used to record temperature, blood pressure, and pump flow rates simultaneously at 5 Hz.

Animal Monitoring and Blood Analysis During Venovenous Perfusion-Induced Systemic Hyperthermia

Blood chemistries, complete blood counts, free hemoglobin, cardiac output, and PA wedge pressure were measured at the following time points: baseline, therapy start, therapy middle (1 hour of 42°C hyperthermia), therapy end (2 hours of 42°C hyperthermia), and cool 39 (when 39°C was achieved). Arterial blood gases and electrolytes were measured every 15 minutes using a blood gas analyzer (Cobas b221, Roche Diagnostics, Indianapolis, Ind). Hemodynamics were continuously monitored, and urine output was measured hourly. Continuous intravenous infusion of lactated Ringer's solution (744 ± 205 mL/h) was used to maintain blood volume for stable hemodynamics. Supplemental calcium chloride (100 mg/mL, IV) or potassium chloride (10-80 mEq, IV) was used as needed to correct hypocalcemia or hypokalemia, respectively. A furosemide bolus (10-50 mg, IV) was given if hourly urine output was less than 50 mL.

Five-Day Post–Venovenous Perfusion-Induced Systemic Hyperthermia Animal Monitoring and Blood Analysis

After completion of the hyperthermia experiment, the sheep were taken off perfusion and the DLC was decannulated. The femoral arterial and Swan–Ganz catheters were left in place throughout the 5-day study for blood pressure monitoring, blood sampling, and fluid administration. The sheep were moved into a metabolic cage and transferred to the intensive care unit. The sheep were weaned from mechanical ventilation/anesthesia. Once spontaneous breathing maintained normal partial pressure of carbon dioxide and partial pressure of oxygen levels, the endotracheal tube was removed. Thereafter, the sheep had free access to food and water throughout the study. For the first 2 days, buprenorphine (0.005-0.02 mg/kg, SC) and enrofloxacin (7.5 mg/kg, SC) were given.

Hemodynamics were continuously monitored and recorded every 6 hours. Blood gases and electrolytes were measured every 6 hours, and complete blood counts/blood chemistries were measured every 24 hours. Neurologic assessments were performed every 24 hours. These assessments were based on the Glasgow Coma scale (0-15), which was modified for sheep to assess cranial nerve function. A score of 15 on this modified Glasgow Coma scale was considered normal.

Animal Euthanasia and Necropsy

After successful completion of the 5-day posthyperthermia survival period, all sheep were euthanized with Beuthanasia-D (1 mL/10 lb body weight, Schering-Plough, Union, NJ) and subjected to a full necropsy. The brain, lungs, heart, liver, kidney, gastrointestinal tract, spleen, adrenals, and bladder were grossly examined, and samples of these tissues were preserved in 10% buffered formalin. The tissue sections were paraffin-embedded and stained with hematoxylin–eosin. Slides were examined by a veterinary histopathology laboratory (Antech Diagnostics, Louisville, Ky).

Data Analysis

All data are expressed as mean ± standard deviation. Differences between the baseline and subsequent time points were evaluated using analysis of variance with Dunnett's post hoc test.

RESULTS

All 5 sheep survived the hyperthermia experiment, achieving 2 hours of 42.0°C ± 0.2°C therapeutic hyperthermia. All sheep recovered from the vv-PISH procedure, were awake with spontaneous breathing within 4 hours, and survived the full 5-day posthyperthermia study period.

Venovenous Perfusion-Induced Systemic Hyperthermia Circuit Efficiency

The baseline core temperature was 38.1°C ± 0.3°C. During the heating phase, a 21 ± 5-minute heating time was needed to achieve a 42°C core temperature. Average warming rate was 1°C per 5.22 ± 0.78 minutes. After the 2 hours of 42°C hyperthermia were completed, 35 ± 6

TABLE 1. Continued

Cool 40	Cool 39	Day 1	Day 2	Day 3	Day 4	Day 5
104 ± 8	101 ± 13	98 ± 9	90 ± 3	95 ± 5	98 ± 9	93 ± 3
160 ± 24*	161 ± 37*	102 ± 6	103 ± 14	104 ± 9	104 ± 9	112 ± 26
16 ± 2	16 ± 1	14 ± 3	15 ± 3	15 ± 3	14 ± 2	15 ± 3
7 ± 3.1	7 ± 3.1	0 ± 2.6	1 ± 3.7	1 ± 2.7	0 ± 1.0	1 ± 1.2
ND	11.5 ± 2.3*	8.2 ± 0.8	7.4 ± 1.2	7.9 ± 1.1	6.9 ± 1.1	7.2 ± 1.3
ND	5 ± 1.1	7 ± 1.5	7 ± 2.4	7 ± 1.5	7 ± 2.3	9 ± 2.3

minutes were taken to cool the sheep to 39°C. Average cooling rate was 1°C per 12.8 ± 1.2 minutes.

Precise Temperature Control of Venovenous Perfusion-Induced Systemic Hyperthermia

The sheep temperature did not exceed 42.5°C at any measured site throughout the hyperthermia experiment (Figure 1, *A*). Moreover, the core temperature (41.8°C-42.2°C) varied by only 0.26°C ± 0.1°C during the 2-hour therapeutic hyperthermia phase (Figure 1, *B*). The circuit blood infusion temperature reached a maximum of 42.3°C ± 0.30°C during the heating phase and a minimum of 36.9°C ± 0.18°C during the cooling phase (Figure 1, *B*).

Hemodynamics

Mean arterial pressure, CVP, mean PAP, and PA wedge pressure were stable and in physiologic range throughout the vv-PISH experiment and 5-day posthyperthermia period (Table 1). Heart rate and cardiac output were significantly increased during the hyperthermia experiment, returning to baseline values by posthyperthermia day 1 and remaining stable throughout the rest of the study.

Fluid and Electrolyte Balance

During the hyperthermia experiment, lactated Ringer's infusion (3070 ± 683 mL) was used to maintain blood volume for stable hemodynamics. Urine output was 292 ± 101 mL/h during vv-PISH. The average daily urine output during the 5 day posthyperthermia study period was 1454 ± 345 mL (72 ± 13 mL/h). Blood sodium levels were within normal physiologic range throughout the vv-PISH and 5-day posthyperthermia monitoring period (Table 2). Blood potassium levels were in normal range during the heating and therapeutic phases, but were lower than physiologic range during the cooling phase. Continuous intravenous potassium chloride supplementation was given during the first 24 hours after vv-PISH, and blood potassium returned to normal levels by 12 hours after vv-PISH. Blood calcium and chloride were in physiologic range throughout the hyperthermia experiment. Bicarbonate and pH were stable during vv-PISH. The pH levels were slightly elevated from post–vv-PISH day 1 to 5 (Table 2).

Hematology

Free hemoglobin levels were significantly increased after instrumentation before starting vv-PISH (baseline, Table 3). Free hemoglobin levels gradually decreased, reaching 7 ± 3 by vv-PISH termination. During the posthyperthermia period, free hemoglobin levels were 3 mg/dL (Table 3). Hematocrit, hemoglobin, and red blood cell counts were unchanged during vv-PISH and within physiologic range during the posthyperthermia study period (Table 4).

White blood cell counts were stable during vv-PISH application but were significantly increased 1 and 2 days after hyperthermia administration, returning to baseline levels by day 3 (Table 4). The percentages of granulocytes, lymphocytes, and monocytes were unchanged (data not shown). Platelet counts were significantly reduced at posthyperthermia days 1 to 3 but were still within normal range.

Blood Comprehensive Metabolism

Blood glucose was elevated slightly above physiologic level during the therapeutic and cooling phases but returned to baseline values by posthyperthermia day 1 (Table 4). Throughout the study, alkaline phosphatase levels were stable. Alanine aminotransferase and aspartate aminotransferase levels were unchanged during vv-PISH, but they were significantly elevated on posthyperthermia days 1 to 3, returning to normal range by day 4. Throughout the study, blood urea nitrogen and creatinine were in the normal range (Table 4). Albumin and total protein were slightly below the normal range during the therapeutic and cooling phases, but returned to baseline values by posthyperthermia day 1. Creatinine phosphokinase (CPK) levels were not significantly altered during vv-PISH application (Table 4). However, CPK levels were elevated 1 and 2 days after hyperthermia, returning to baseline values by posthyperthermia day 3.

Neurologic Assessment

All 5 sheep scored 15 on the Glasgow Coma scale throughout the posthyperthermia period, indicating normal cranial nerve function. The sheep exhibited normal behavior in terms of eating, standing, and response to humans.

Histology

No abnormalities were observed in the brain, heart, and adrenal glands of all 5 sheep. The lungs in all sheep had areas of atelectasis that were likely due to previous lung infection or anesthetic procedure. Mild diffuse hepatocellular cytoplasmic clumping and coagulation were found in the livers of all 5 sheep, an effect likely due to mild hypoxia. Two sheep had 1 kidney exhibiting signs of ischemic injury, whereas the other kidney was normal. The stomach in 3 sheep showed signs of gastritis. Inflammation with coexisting parasitism was present in the intestinal tract of all 5 sheep. Spleen congestion also was found in 4 sheep.

DISCUSSION

In this 5-day GLP-compliant sheep survival study, our simplified vv-PISH system safely delivered an accurate therapeutic hyperthermia dose with no evidence of immediate or long-term hyperthermia-related complications. With our vv-PISH system, fast heating with stable ABP/PAP/CVP, normal to near normal levels of blood electrolytes/metabolic enzymes/hematologic parameters, and negligible vv-PISH–related hemolysis were achieved with rapid anesthesia recovery.

The vv-PISH system uses an extracorporeal pump-heat exchanger circuit to withdraw a portion of venous blood for heating, which is then pumped back into the pulmonary system where it is well mixed with unheated venous blood. This heated blood from the pulmonary circulation is then evenly distributed to the systemic circulation by the left side of the heart to achieve homogenous heat delivery.[16,18,21] With the vv-PISH system, hyperthermia can be delivered to all sites affected by metastatic cancer, including the visceral organs and previously privileged areas for cancer metastasis, such as the bone marrow, brain, vertebral column, and mediastinum.[16,18] Another advantage of the vv-PISH system is the shorter heat time needed to reach the target temperature, which enhances cancer cell kill by limiting the recruitment of thermoprotective mechanisms.[8,12,18,21,24] Moreover, rapid heating expedites apoptosis to more efficiently kill cancer cells.[8,24] Thus, the vv-PISH system has significant advantages that will likely enhance the efficacy of hyperthermia.

A first-generation vv-PISH circuit was developed by our group and tested in 10 patients with advanced NSCLC in a phase I safety trial with promising results.[16-18] A DLC-based extracorporeal circuit for systemic hyperthermia also has been developed by an Austrian group.[25] Although these systemic hyperthermia systems functioned adequately in their respective clinical trials, there were several critical issues requiring resolution. First, unstable hemodynamics in all the patients required norepinephrine, large volume fluid resuscitation (6 L), and vigorous diuresis.[18,25] Second, posthyperthermia somnolence existed for up to 48 hours, and extubation was delayed up to 36 hours because of pulmonary edema.[17,18] Third, moderate hemolysis and thrombocytopenia were observed.[17,25] Fourth, the heating phase was too long (>45 minutes), allowing time for the cancer cells to develop thermotolerance.[17,18,25]

To address these issues, we simplified the vv-PISH circuit and established anesthesia/circuit management protocols to prevent arterial hypotension and pulmonary hypertension.[20,21] In the current study, this next-generation vv-PISH circuit successfully delivered an accurate hyperthermia dose. Hemodynamics were stable with no need for vasopressors or large fluid volumes. All sheep quickly recovered from anesthesia within 4 hours of vv-PISH termination. Neurologic assessments were normal in all sheep throughout the posthyperthermia study period, indicating the absence of brain injury. There was no vv-PISH circuit-related hemolysis as indicated by free hemoglobin levels less than 20 during and after vv-PISH use. Free hemoglobin levels were elevated after instrumentation but before vv-PISH circuit hook-up, an effect most likely due

TABLE 2. Electrolytes

	Baseline	Therapy start	Therapy middle	Therapy end	Cool 41	Cool 40	Cool 39	Day 1	Day 2	Day 3	Day 4	Day 5
Na^+	141 ± 1	139 ± 2	137 ± 2*	136 ± 1*	136 ± 1*	136 ± 2*	135 ± 2*	147 ± 2*	147 ± 1*	145 ± 1*	145 ± 2*	146 ± 2*
K^+	3.52 ± 0.43	4.01 ± 0.38	3.93 ± 0.42	3.74 ± 0.20	3.33 ± 0.27	3.13 ± 0.17	3.21 ± 0.30	3.96 ± 0.27	4.04 ± 0.13	3.87 ± 0.2	3.92 ± 0.27	3.95 ± 0.37
Ca^{2+}	1.15 ± 0.07	1.09 ± 0.02	1.11 ± 0.04	1.18 ± 0.14	1.12 ± 0.06	1.09 ± 0.06	1.15 ± 0.08	1.22 ± 0.03	1.25 ± 0.07	1.22 ± 0.10	1.26 ± 0.05	1.26 ± 0.04
Cl^-	102 ± 2	103 ± 1	100 ± 2	99 ± 1	101 ± 1	101 ± 2	100 ± 2	110 ± 3*	104 ± 2	106 ± 5	104 ± 2	105 ± 2
$HCO3^-$	27.9 ± 0.7	26.4 ± 1.2	26.9 ± 0.8	27.0 ± 0.5	25.6 ± 0.7	25.1 ± 0.9	25.9 ± 1.4	21.8 ± 2.0*	27.4 ± 2.1	25.4 ± 4.0	24.5 ± 1.7*	24.3 ± 2.3*
pH	7.41 ± 0.07	7.39 ± 0.03	7.40 ± 0.03	7.41 ± 0.03	7.42 ± 0.01	7.41 ± 0.02	7.41 ± 0.02	7.46 ± 0.04	7.48 ± 0.02*	7.48 ± 0.01*	7.51 ± 0.02*	7.49 ± 0.01*

Arterial blood electrolytes were measured at baseline, therapy start (when 42°C target was met), therapy middle (1 hour at 42°C), therapy end (2 hours at 42°C), cool 41 (core temperature 41°C during cooling), cool 40 (core temperature 40°C during cooling), cool 39 (core temperature 40°C during cooling), day 1 (posthyperthermia day 1), day 2 (posthyperthermia day 2), day 3 (posthyperthermia day 3), day 4 (posthyperthermia day 4), and day 5 (posthyperthermia day 5). N = 5 sheep. Ca^{2+}, Calcium; Cl^-, chloride; $HCO3^-$, bicarbonate; K^+, potassium; Na^+, sodium. *$P < .05$ versus baseline.

TABLE 3. Free hemoglobin

Sheep	Presurgery	Baseline	Therapy start	Therapy middle	Therapy end	Cool 39	Day 1	Day 2	Day 3	Day 4	Day 5
1	7	17	INT	INT	INT	INT	4	3	3	3	3
2	7	20	10	3	3	11	3	3	3	3	3
3	4	25	13	7	6	4	3	4	3	3	3
4	7	43	20	13	10	7	3	3	3	3	3
5	7	43	18	16	13	7	3	3	3	3	3
Mean	6 ± 1.3	30 ± 13*	15 ± 5	10 ± 6	8 ± 4	7 ± 3	3 ± 0.4	3 ± 0.5	3 ± 0.0	3 ± 0.0	3 ± 0.0

Free hemoglobin levels were measured at presurgery (before surgery day), baseline (after instrumentation but before vv-PISH start), therapy start (when 42°C target was met), therapy middle (1 hour at 42°C), therapy end (2 hours at 42°C), cool 39 (when 39°C baseline temperature was restored), day 1 (posthyperthermia day 1), day 2 (posthyperthermia day 2), day 3 (posthyperthermia day 3), day 4 (posthyperthermia day 4), and day 5 (posthyperthermia day 5). *INT*, Interference due to severe lipemia in blood sample. *$P < .05$ versus presurgery.

to cannulation-induced hemolysis. In the current study, thrombocytopenia was not observed. With our vv-PISH circuit, fast heating while maintaining hemodynamic stability was possible, resulting in a shorter heating time (21 ± 5 minutes) to reach the 42°C core temperature. This is an important finding because hyperthermia induction must be rapid enough to limit thermotolerance but slow enough to maintain patient stability.[26]

In this 5-day sheep survival study, there were no severe hyperthermia-related complications, indicating the safety of our vv-PISH system. Slight elevations in liver enzymes were detected 1 to 3 days after hyperthermia treatment, but their return to normal range suggests the absence of significant liver injury. Two sheep had an ischemic injury to one of their kidneys. Given the normal renal performance during the study, and only 1 kidney being involved, it is unlikely to be related to the systemic hyperthermia treatment. Nevertheless, renal and hepatic function will be closely monitored during future patient hyperthermia treatments. CPK levels were elevated 5-fold 1 day after hyperthermia administration, but returned to normal range by day 3. A 39-fold increase in CPK levels also was observed in the previous vv-PISH clinical trial, and the clinical study by the Austrian group also found elevations in this enzyme.[17,25] Because the release of CPK from skeletal muscle occurs during a malignant hyperthermia episode,[27,28] the increase in CPK seems to be a normal physiologic response to hyperthermia.

CONCLUSIONS

In this preclinical GLP study, our vv-PISH system safely and reliably delivered the therapeutic hyperthermia dose with no hyperthermia-related complications. We have planned the following 3 steps toward clinical application of vv-PISH: (1) a Food and Drug Administration investigational device exemption for human trial (in process), (2) an institutional review board–approved clinical trial to prove the safety of vv-PISH in humans, and (3) a controlled clinical trial to evaluate the efficacy of vv-PISH in lung cancer treatment.

TABLE 4. Hematology and blood chemistry

Parameter	Baseline	Therapy start	Therapy middle	Therapy end	Cool 39	Day 1	Day 2	Day 3	Day 4	Day 5
Hematocrit (%)	26 ± 1	27 ± 0.4	27 ± 0.7	26 ± 1.1	25 ± 2	30 ± 1*	30 ± 3*	27 ± 2	28 ± 1	26 ± 3
Hemoglobin (g/dL)	9.1 ± 0.5	8.9 ± 0.5	9.0 ± 0.3	8.7 ± 0.5	8.5 ± 0.7	10.4 ± 0.6*	10.5 ± 1*	9.7 ± 0.8	9.9 ± 0.4	9.4 ± 0.7
RBC ($\times 10^6/\mu L$)	9.8 ± 0.7	9.3 ± 0.6	9.3 ± 0.6	8.9 ± 0.6	8.6 ± 0.8	11.2 ± 0.9	11.3 ± 1.4	10.3 ± 1	10.6 ± 0.9	10.0 ± 0.9
WBC ($\times 10^3/\mu L$)	5.5 ± 1.9	7.1 ± 2.2	7.5 ± 2.4	6.6 ± 2.3	6.1 ± 2.7	12.0 ± 3.7*	10.3 ± 2.7*	8.4 ± 1.6	7.7 ± 2.1	6.9 ± 1.2
Platelets ($\times 10^3/\mu L$)	527 ± 59	571 ± 40	588 ± 8	546 ± 42	496 ± 54	335 ± 70*	363 ± 47*	393 ± 67*	481 ± 102	462 ± 97
Glucose (mg/dL)	75 ± 17	101 ± 4*	111 ± 17*	140 ± 21*	147 ± 19*	79 ± 8	82 ± 11	82 ± 5	84 ± 16	89 ± 6
ALP (IU/L)	158 ± 40	168 ± 36	166 ± 36	161 ± 31	157 ± 32	162 ± 35	156 ± 54	130 ± 29	118 ± 19	121 ± 26
ALT (IU/L)	12 ± 4	11 ± 4	11 ± 4	10 ± 3	11 ± 4	70 ± 33*	61 ± 26*	46 ± 20*	32 ± 15	31 ± 13
AST (IU/L)	71 ± 9	63 ± 5	67 ± 5	68 ± 4	71 ± 7	377 ± 127*	301 ± 72*	205 ± 47*	148 ± 33	142 ± 19
BUN (mg/dL)	18 ± 3	17 ± 2	18 ± 3	17 ± 3	17 ± 3	7 ± 2*	10 ± 2*	10 ± 2*	11 ± 3*	13 ± 3*
Creatine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Albumin (g/dL)	2.8 ± 0.2	2.4 ± 0.1*	2.4 ± 0.1*	2.3 ± 0.1*	2.2 ± 0.2*	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.2	3.0 ± 0.2	2.9 ± 0.2
Total protein (g/dL)	5.3 ± 0.5	4.4 ± 0.2*	4.2 ± 0.2*	4.0 ± 0.2*	3.9 ± 0.3*	5.5 ± 0.2	5.5 ± 0.2	5.4 ± 0.1	5.7 ± 0.2	5.5 ± 0.2
CPK (IU/L)	213 ± 16	266 ± 27	336 ± 29	427 ± 83	572 ± 184	1118 ± 1056*	458 ± 317	196 ± 80	168 ± 93	153 ± 83

Complete blood counts were measured at baseline, therapy start (when 42°C target was met), therapy middle (1 hour at 42°C), therapy end (2 hours at 42°C), cool 39 (when 39°C baseline temperature was restored), day 1 (posthyperthermia day 1), day 2 (posthyperthermia day 2), day 3 (posthyperthermia day 3), day 4 (posthyperthermia day 4), and day 5 (posthyperthermia day 5). N = 5 sheep. *ALP*, Alkaline phosphatase; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *BUN*, blood urea nitrogen; *CPK*, creatinine phosphokinase; *RBC*, red blood cell; *WBC*, white blood cell. *$P < .05$ versus baseline.

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